AD NUMBER

AD800591

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; SEP 1966. Other requests shall be referred to Department of the Army, Fort Detrick, Frederick, MD 21701.

AUTHORITY

Army Biological Defense Research Lab ltr dtd 28 Sep 1971

TECHNICAL MANUSCRIPT 315

ELIMINATION OF PLEUROPNEUMONIA-LIKE ORGANISMS FROM TISSUE CULTURE

Roger W. Johnson
Michael D. Orlando

SEPTEMBER 1956

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 315

ELIMINATION OF PLEUROPNEUMONIA-LIKE ORGANISMS FROM TISSUE CULTURE

Roger W. Johnson

Michael D. Orlando

Process Development Division
AGENT DEVELOPMENT AND EVALUATION LABORATORY

Project 1B533601D426

September 1966

ELIMINATION OF PLEUROPNEUMONIA-LIKE ORGANISMS FROM TISSUE CULTURE

ABSTRACT

Tissue cultures heavily contaminated with PPLO were treated with kanamycin (1 mg/ml of tissue culture medium for 48 hours), kanamycin in combination with heat (41 C for 18 hours) or novobiocin (50 mg/ml of tissue culture medium for 7 days) in combination with heat. Only treatment with novobiocin and heat was successful in ridding the cultures of the PPLO contamination.

Pleuropneumonia-like organisms (PPLO) are the crabgrass of tissue culture. Many reports in the literature document successful attempts to eliminate these organisms from tissue cultures, but usually such reports were stimulated by the failure of some previously described method to be successful in the hands of the investigator.

Although our cultures exhibited no overt manifestations of contamination, upon culture for PPLO we found that they were heavily contaminated with PPLO. This report describes the successful elimination of PPLO from our tissue cells by a combination of treatment with novobiocin[1] and heat.[2]

Table 1 describes the treatments to which monolayer cultures of L (Earle) cells, grown in 32-oz prescription bottles, were subjected in attempts to eliminate the PPLO. After the treatments the cultures were fed fresh growth medium (medium 199 plus 10% calf serum) and were grown free of antibiotics. Cultures were tested for PPLO about 14 days after treatment, and at periodic intervals thereafter.

PPLO were detected by growth on the agar medium described by Chanock, Hayflick, and Barile[3] modified as follows: Bacto yeast extract was used in place of an extract of baker's yeast, and dextrose (10 g/liter) was added. Inoculated plates were incubated at 37 C in an atmosphere of 95% N_2 and 5% CO_2 for 14 days. A known culture of PPLO was inoculated each time as a positive control.

The results of these experiments are shown in Table 2. Neither kanamycin nor heat treatment alone was capable of ridding the cultures of PPLO. PPLO could not be isolated from cultures treated with a combination of kanamycin and heat 14 days after this treatment. However, after 2 or 3 serial transfers of the cells PPLO could again be detected by culture. Only treatment with novobiocin and heat was effective in ridding the cultures of PPLO. These cultures are still negative for PPLO 8 months after treatment. Another investigator* was unable to eliminate PPLO from the contaminated cells using novobiocin and kanamycin but no heat.

---

* Richard C. Carter, personal communication.

TABLE 1. TREATMENTS ATTEMPTED TO ELIMINATE PPLO FROM MONOLAYER TISSUE CULTURES[a]

Treatments	Antibiotic Level	Antibiotic Exposure Time	Heat 41 C Exposure Time
1. Kanamycin[b]	1 mg/ml	48 hr	-
2. Heat	-	-	18 hr
3. Kanamycin plus heat	1 mg/ml	48 hr	18 hr
4. Heat plus kanamycin	1 mg/ml	48 hr	18 hr
5. Novobiocin[c] plus heat	50 µg/ml	7 days	18 hr

a. Volume of growth medium (medium 199 supplemented with 10% calf serum) was 50 ml for all treatments.
b. Kantrex®, Bristol Laboratories, Syracuse, N. Y.
c. Cathomycin®, Merck, Sharp and Dohme, West Point, Pa.

TABLE 2. PPLO ISOLATIONS FROM CONTAMINATED CULTURES FOLLOWING ANTIBIOTIC OR HEAT TREATMENT

Treatment	Days after Treatment 14	28	240
Kanamycin (1 mg/ml)[a]	Pos		
Heat (41 C for 18 hr)	Pos		
Kanamycin followed by heat	Neg	Pos	
Heat followed by kanamycin	Neg	Pos	
Novobiocin (50 µg/ml)[b] followed by heat	Neg	Neg	Neg
Control	Pos	Pos	Pos

a. Kantrex®, Bristol Laboratories, Syracuse, N. Y.
b. Cathomycin®, Merck, Sharp and Dohme, West Point, Pa.

That PPLO are difficult to eliminate from tissue culture is obvious from the number of methods described in the literature for their elimination. It appears that not all strains of PPLO are susceptible to the same treatments. The failure to eliminate PPLO from our cultures by either kanamycin or heat alone, techniques successful in the hands of others,[2,4] supports this view. The reappearance of PPLO in the cultures treated with both kanamycin and heat suggests that these organisms in their life cycle might be protected in some way from this adverse environment and might remain dormant for a period.

It is unlikely that the reappearance of PPLO in these cultures represents reinfection because (i) the treated cultures were handled only after swabbing the working area with hypochlorite solution, and prior to handling untreated cultures, and (ii) PPLO failed to appear in the cultures treated with novobiocin and heat. If PPLO were to appear now in the cultures treated with novobiocin and heat they would probably have arisen from an exogenous source.

Treatment of our cultures with novobiocin and heat suffers from its being a "shotgun" treatment. However, our primary concern was to eliminate PPLO from our cultures, and this we were successful in doing by this technique.

Although selection cannot be ruled out, the growth characteristics and morphology of the surviving cells, and their ability to produce virus, did not appear to be altered by the treatment.

LITERATURE CITED

1. Balduzzi, P.; Charbonneau, R.J. 1964. Decontamination of pleuropneumonia-like organism (PPLO) infected tissue culture. Experientia 20:651-652.

2. Hayflick, Leonard. 1960. Decontaminating tissue cultures infected with pleuropneumonia-like organisms. Nature 185:783-784.

3. Chanock, R.M.; Hayflick, L.; Barile, M.F. 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Nat. Acad. Sci. 48:41-49.

4. Foch, J.; Hacker, C. 1960. Elimination of pleuropneumonia-like organisms from cell cultures. Exp. Cell. Res. 21:242-244.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) DEPARTMENT OF THE ARMY Fort Detrick Frederick, Maryland 21701	2a REPORT SECURITY CLASSIFICATION Unclassified 2b GROUP

3 REPORT TITLE

ELIMINATION OF PLEUROPNEUMONIA-LIKE ORGANISMS FROM TISSUE CULTURE

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)

Johnson, Roger W.
Orlando, Michael D.

6 REPORT DATE September 1966	7a TOTAL NO OF PAGES 8	7b NO OF REFS 4
8a CONTRACT OR GRANT NO b PROJECT NO 1B533601D426 c d	9a ORIGINATOR'S REPORT NUMBER(S) Technical Manuscript 315 9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	

10 AVAILABILITY/LIMITATION NOTICES

Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY DEPARTMENT OF THE ARMY Fort Detrick Frederick, Maryland 21701

13 ABSTRACT

Tissue cultures heavily contaminated with PPLO were treated with kanamycin (1 mg/ml of tissue culture medium for 48 hours), kanamycin in combination with heat (41 C for 18 hours) or novobiocin (50 mg/ml of tissue culture medium for 7 days) in combination with heat. Only treatment with novobiocin and heat was successful in ridding the cultures of the PPLO contamination.

14. Key Words

*Mycoplasma	Kanamycin
*Tissue cultures	L cells
Novobiocin	Culture media
Heat	

DD FORM 1 JAN 66 1473

Unclassified

Security Classification